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The world's smallest Campanulaceae: *Lysipomia mitsyae* sp. nov.

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Abstract Botanists and plant morphologists have long been fascinated by how certain species can exhibit such reduced morphologies that even their identification to genus- or family-level becomes difficult. Such was the case with *Lysipomia mitsyae* sp. nov., an exceptionally small plant discovered in the Peruvian Andes which bears lobelioid characteristics but differs in size by an order of magnitude from the current smallest members known from the entire Campanulaceae and lacks diagnostic characters allowing it to be reliably placed to genus-level. Molecular analyses of *trnL-F*, composed of a representative Lobelioideae sampling, place the samples within the genus *Lysipomia*, requiring that amendments be made to the description of the genus. Supplementary ITS analyses of a representative generic sampling indicate a close relationship to *Lysipomia sphagnophila* and *L. multiflora*. We here describe the world's smallest Campanulaceae, *Lysipomia mitsyae* sp. nov., and discuss its phylogenetic and systematic relationships to the other members of the genus. Its highly reduced morphology, which has given it status as the smallest Campanulaceae and, quite possibly, the world's smallest eudicot, is discussed in the light of current knowledge on the physiological and anatomical constraints on alpine plant growth and survival.

Keywords alpine; Andes; Bayesian analysis; dwarfism; Lobelioideae; smallest eudicot

Supplementary Material DNA sequence alignment is available from TreeBASE (study nos. 19056 and 19057).

■ INTRODUCTION

The world's smallest known angiosperms belong to the genus *Wolffia* Horkel ex Schleid. of the monocot family Araceae. Within eudicots there are a number of contenders for the title of "smallest" species and, interestingly, the majority of these are found in high-elevation habitats. The smallest eudicot is commonly believed to be the dwarf mistletoe *Arceuthobium minutissimum* Hook.f., a leafless parasite of pine trees in the Himalayan altimontane zone measuring a mere 2–5 mm (Datta, 1951). However, during plant surveys in the heavily grazed puna grasslands of the Cordillera Urubamba, southern Peru (Heitkamp & al., 2014), we discovered an annual autotrophic plant measuring just 1.8–4(–5.5) mm in height that represents another contender for the world's smallest eudicot.

The plants collected bear characteristics of Campanulaceae (e.g., connate lobelioid-like androecium surrounding the style and adnate to the corolla tube, and inferior ovaries) but differ in size by an order of magnitude from the current smallest members known from the family. These diminutive plants exhibit a morphology that did not fit any of the genera currently recognised, initially hampering a reliable generic placement based on morphological characters alone. Campanulaceae is a diverse family comprising 84 genera and ca. 2400 species that occur in a vast array of habitats and exhibit a great diversity of life-forms ranging from dwarf herbs to 15 m tall trees (Lammers, 2007; Antonelli, 2008). Morphologically, these plants are most similar to species of the genus *Lysipomia* Kunth, but with several important differences. *Lysipomia*, until now, has been considered to comprise ca. 30–40 species of usually perennial low-rosulate cushion-forming herbs with congested alternate leaves that measure more than 10 mm and obviously bilabiate flowers (Wimmer, 1937, 1953; McVaugh, 1955; Jeppesen, 1981; Ayers, 2000). The species described here is a diminutive short-lived monocarp with apparently opposite leaves, due to the prominent persistent cotyledons and rare occurrence of true leaves, both which measure less than 5 mm long, and weakly bilabiate flowers that emerge from the axils of the cotyledons. To resolve the placement of this species within Campanulaceae, we conducted a phylogenetic analysis to determine the systematic relationships of the plant.

In this paper, using Bayesian inference and maximum likelihood analyses based on plastid *trnL-F* and nuclear ITS data, we place the new lobelioid species from the high Andean puna grasslands in *Lysipomia* and examine its relationship within the genus. We describe and illustrate the new species, and present an updated phylogeny for *Lysipomia*. We then discuss the systematic relationships of this new species to the other members of the genus and its highly reduced morphology, which gives it status as the world's smallest Campanulaceae.

■ MATERIALS AND METHODS

Taxon sampling and molecular markers. — Our study was based on specimen collections *S.P. Sylvester 823* and *1417* that

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were distanced by ca. 600 m from each other. For the species description, ca. 500 individual plants, pertaining to both collections, were morphologically studied. Extreme range size values mentioned in the species description relate to less than 10 specimens in which these sizes were observed. Individual plants from both specimen collections were sequenced for the phylogenetic analyses.

From the beginning, it was clear that the plant material morphologically belonged to Campanulaceae subfamily Lobelioideae due to its distinct floral morphology, but its precise placement within the subfamily was unclear. Therefore, we compiled a Lobelioideae backbone dataset of *trnL-F* sequences from GenBank, including the *trnL*^{UAA} group I intron and the *trnL-trnF* intergenic spacer (IGS), guided by Antonelli (2008) and Lagomarsino & al. (2014) (see Appendix 1). In addition to the downloaded *trnL-F* sequences, we added sequence data for two accessions included in the morphological analyses (*S.P. Sylvester 823* and *1417*) as well as *L. sphagnophila* subsp. *sphagnophila* Griseb. ex Wedd, *L. laciniata* A.DC., and *L. glandulifera* (Schltdl. ex Wedd.) Schltdl. ex E.Wimm. (see Appendix 1). As *trnL-F* analyses placed both accessions in *Lysipomia*, an independent second dataset comprising the nrITS region, including the internal transcribed spacers (ITS) 1 and 2 as well as the 5.8S gene of the nuclear ribosomal DNA (compare Wicke & al., 2011) was compiled to pinpoint the relationship of our specimens with other species of *Lysipomia*. Fortunately, ITS sequence data for a large proportion of known *Lysipomia* species was already present in GenBank from a study by Ayers (1999) that served as a basis for the ITS dataset. In addition to sequences from a representative sampling of the paraphyletic *Lobelia* L. (Antonelli, 2008), we included representatives from *Burmeistera* H.Karst & Triana and *Centropogon* C.Presl in the ITS dataset as further outgroups (cf. Antonelli, 2008).

Molecular methods. — Whole genomic DNA of each individual specimen was isolated using commercially available spin columns (NucleoSpin Plant II, Macherey-Nagel, Düren, Germany) following the manufacturer's protocol. Prior to incubation with the lysis buffer, plant material was pulverized in 2 ml Eppendorf caps (round bottom) containing 3 glass beads (5 mm) with a Mixer Mill (Retsch TissueLyser, Qiagen, Hilden, Germany) at 30 Hz (two rounds of 1 min). PCR amplifications (T3 Thermocycler, Biometra, Göttingen, Germany) were performed in 25 µl-reactions containing 1 U Taq-DNA polymerase (GoTaq, Promega, Mannheim, Germany), 1 mM dNTP mix of each 0.25 mM, 1× buffer, 1.25–2.5 mM $MgCl_2$ and 20 pmol of each amplification primer. PCR protocols for the *trnL-F* region followed Borsch & al. (2003). The ITS region was amplified using the primers ITS4 and ITS5 designed by White & al. (1990) with an amplification profile of: 5 min 94°C, 40 cycles (1 min 94°C, 1 min 48°C with a time-increment of +4 s/cycle, 45 s 68°C) and a final extension of 7 min at 68°C. Column cleaned PCR products (NucleoSpinR Gel and PCR Cleanup, Macherey-Nagel) were sequenced by Macrogen, South Korea (http://www.macrogen.com). Quality control of the pherograms, elimination of primer sequences and contig assembly was done in PhyDE v.0.996 (available at http://www.phyde.de).

Phylogenetic reconstructions. — Alignment of the sequences was performed manually with PhyDE v.0.996, based on the criteria laid out in Kelchner (2000) for motif alignments. Indels were incorporated in the phylogenetic reconstructions employing a simple indel coding (SIC) approach as advocated by Simmons & Ochoterena (2000) using the PAUP command file generated by Seqstate v.1.4.1 (Müller, 2005, 2006). Maximum likelihood (ML) reconstructions were done using RAxML (Stamatakis, 2014) applying the GTRGAMMA model for nucleotide evolution and F81 for indels. Internal branch support was estimated by heuristic bootstrap (BS) searches with 10,000 replicates each. Bayesian analyses were performed with MrBayes v.3.2.5 (Ronquist & al., 2012), applying the GTR+Γ+I model. Four runs with four chains (5×10^6 generations each) were run simultaneously, with a chain sampling every 1000 generations. Tracer v.1.6 (Rambaut & al., 2014) was used to examine the log likelihoods to determine the effective sampling size and stationarity of the MCMC search. Calculations of the consensus tree, including clade posterior probability (PP), were performed based on the trees sampled after the chains converged (below generation 500,000). Consensus topologies and support values from the different methodological approaches were compiled and drawn using TreeGraph v.2 (Stöver & Müller, 2010).

■ RESULTS

Taxonomic treatment

Lysipomia mitsyae Sylvester & D.Quandt, **sp. nov.** – Holotype: PERU. Cuzco, Provincia Calca, Distrito Calca, grazed ground below the SW facing crags of the Laguna Yanacocha, 1.5 km E of Cancha Cancha village, Huarán, 4340 m, 13°14′28.4″ S, 72°01′16.8″ W, 27 Mar 2012 (fl and fr), *S.P. Sylvester 1417* (USM No. USM285670; isotypes: CUZ, K barcode K000501660, LPB No. LPB0000943, Z barcode Z-000099206). — Fig. 1.

Diagnosis. – Differs from all other species of *Lysipomia* by being an extremely small ephemeral plant less than 5.5 mm tall, with prominent persistent cotyledons measuring <5 mm and a frequent absence of smaller true leaves, with only 1–3 flowers emerging from the cotyledon axils, and presence of staminodes.

Description. – Minute glabrous short-lived monocarpic *herb*, 1.8–4(–5.5) mm tall. *Stem* 0.3–2 mm long. *Cotyledons* persistent, subsessile, elliptic, 1–2.6(–5) mm long, 0.4–0.6 mm wide, slightly convex, apically obtuse, glabrous, marginally slightly thickened, slightly glassy, entire, the blades attenuate into short petioles much shorter than the blades, basally slightly broadened to sheathing. *Leaves* absent or 1(–2) sometimes present above the cotyledons, alternate but appearing opposite due to the prominent cotyledons, crowded and imbricate with the cotyledons, usually arising at 90° angle with the cotyledons and appearing decussate when 2 leaves present, subsessile, elliptic, usually shorter than the cotyledons, 0.5–1.5(–5) mm long, 0.25–0.5 mm wide, flat, apically obtuse, glabrous, marginally slightly thickened, slightly glassy, entire, the blades attenuate into short petioles much shorter than the blades, basally slightly broadened

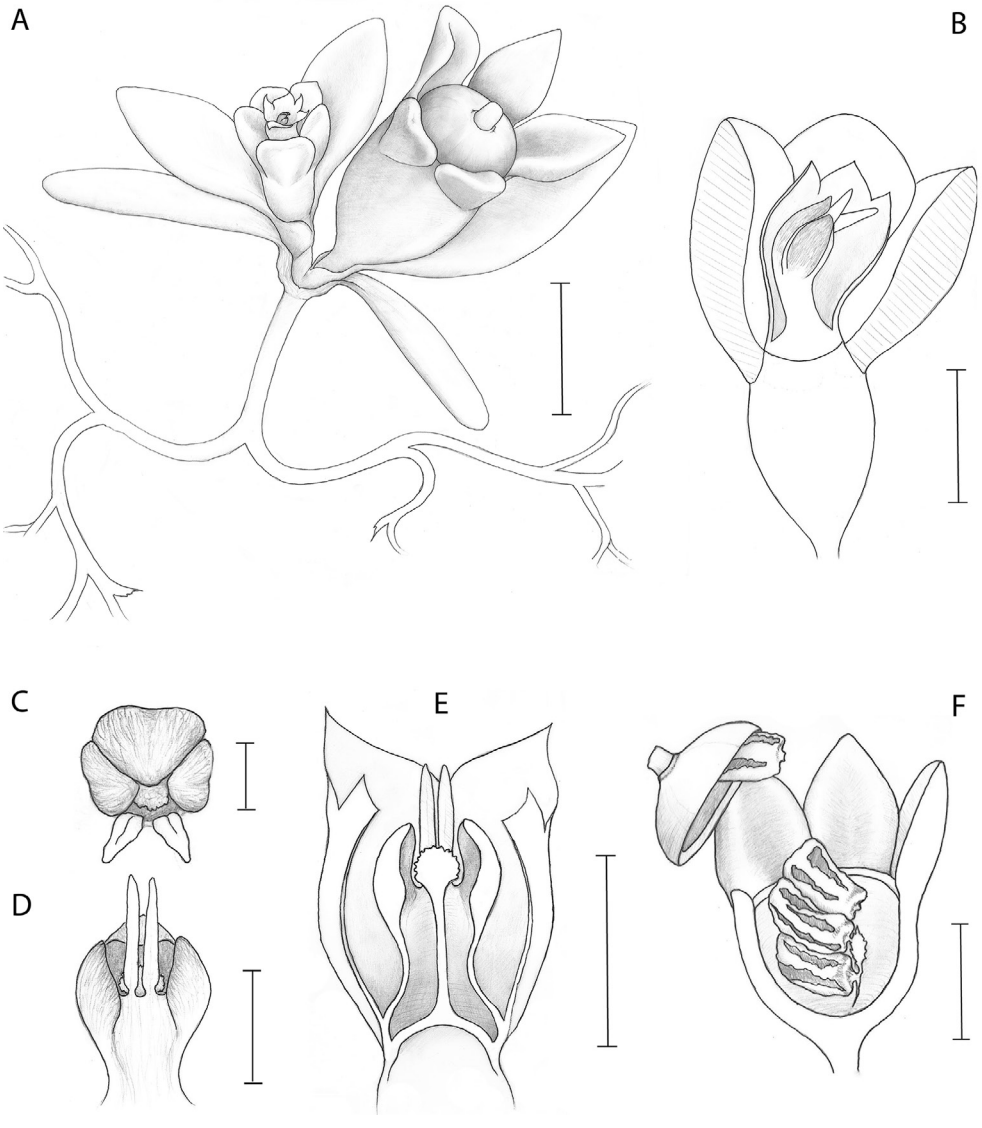


Fig. 1. *Lysipomia mitsyae* Sylvester & D.Quandt, sp. nov. **A,** Habit; **B,** Side-view of a flower with petals and sepals removed to reveal the androecium; **C,** Front view of the androecium, note the lower staminodial flap-like appendages; **D,** Ventral view of the androecium with staminodial appendages in the foreground; **E,** Longitudinal section of the corolla and androecium to reveal the style and stigma; **F,** Longitudinal section of a capsule, showing the operculum and seeds attached to the thin placental intrusion on the ventral surface of the ovary. — Scale bars: A & F = 1 mm, B & E = 0.5 mm, C & D = 0.2 mm. Illustrations by Lolita Ammann based on *S.P. Sylvester 1417.*

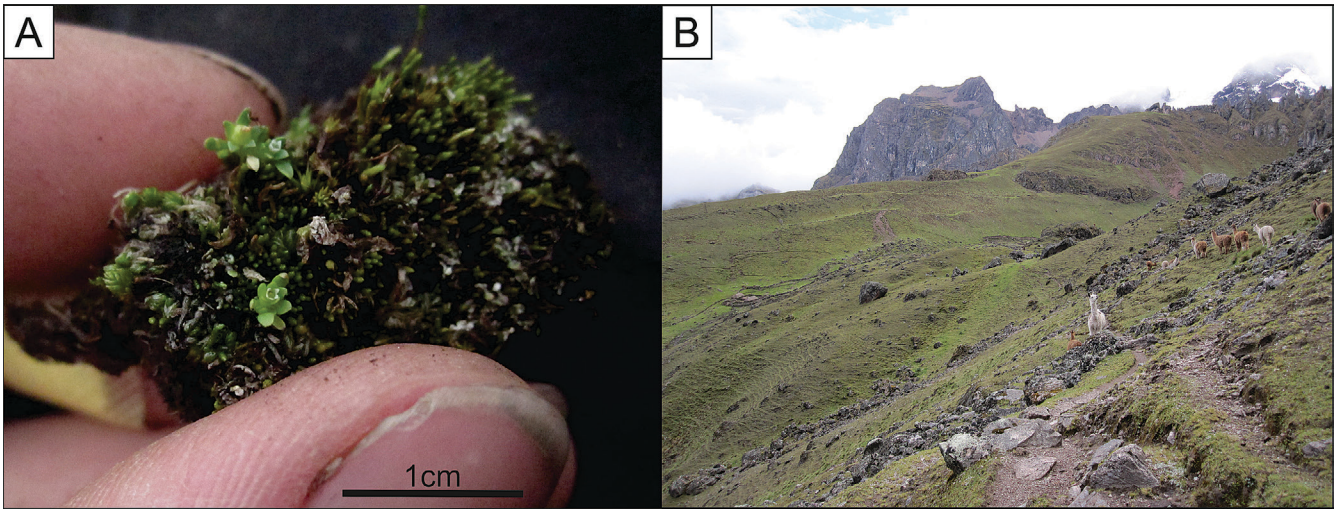


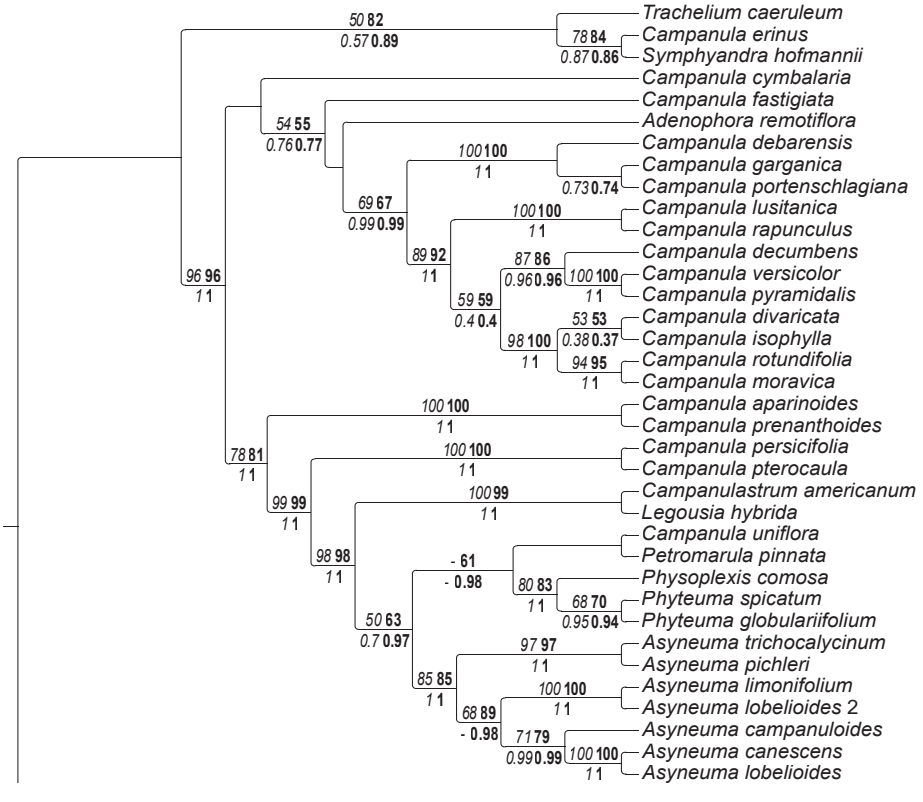
Fig. 2. A, *Lysipomia mitsyae* showing fruiting and flowering specimens; **B,** Habitat of *L. mitsyae*: heavily grazed puna grassland. — Photos by S.P. Sylvester.

or not broadened at all. *Flowers* emerging from cotyledon axils, 1(–3) per plant, 1.5–2.5(–3.3) mm long, subsessile, pedicels 0.05–0.15 mm long. *Calyx* with sepals obtuse, subequal, the dorsal sepal 0.8–1.3 mm long, the two lateral sepals slightly shorter, 0.7–1.0 mm long, the two ventral sepals (0.4–)0.6–0.8 mm long, apex obtuse, margin entire, eglandular, persisting in fruit. *Corolla* white, tube 0.5–0.8 mm long to the lateral sinus, limbs faintly bilabiate, the lip presented horizontally or vertically facing the stem apices, the dorsal lobes broadly lanceolate, ca. 0.2 mm long, varying from slightly deflexed to incurved, the ventral lobes broadly lanceolate, ca. 0.1–0.18 mm long, varying from slightly deflexed to incurved. *Filament tube* equal to corolla tube. *Anther tube* black, 0.2–0.3 mm long, not exerted from the corolla, the upper 3 anthers functional, the lower 2 staminodial and present as hyaline lobes emerging ventrally from the stamen tube, ca. 0.2 mm long. *Hypanthium* turbinate, 0.5–0.95 mm long, smooth or minutely winged at ribs when dry. *Ovaries* unilocular, placentation parietal, ovules in 2 rows inserted on a thin placental intrusion on the ventral surface. *Capsules* turbinate, ca. 1–2.2 mm long, (0.5–)1–1.5 mm wide, unilocular. *Seeds* ca. 8 per capsule, ovoid to slightly kidney-shaped, narrowing slightly to a prominent circular hilum, ca. 0.5 mm long, dark brown, with irregular longitudinal furrows and nearly smooth surface with indistinct cross-lines. See Figs. 1A–F and 2A. Chromosome number unknown.

Ecology and distribution. – Locally abundant and common on bare ground open to sunlight in heavily grazed puna grassland (Fig. 2B). Up to 500 individual plants were encountered in a single 2 × 2 m² plot studied at the type locality and indicator species analyses have retrieved this species as a significant indicator species of grazed puna grassland (Sylvester & al., unpub. data). During fieldwork from August 2010 to June 2012, the species was only found in the vegetation plots in March–May, with visits to the plots in June–October being unsuccessful in encountering the species. This confirms *L. mitsyae* to be ephemeral, with it likely to establish and flower towards the end of the rainy season before the onset of cold and dry conditions from June to October. The plants were found to root in a hardened organic soil crust and were found associated with mosses, lichens and other low-growing plants such as *Agrostis breviculmis* Hitchc., *Azorella biloba* (Schltdl.) Wedd. and *Lachemilla pinnata* (Ruiz & Pav.) Rothm. The species is currently known only from two sites in the same valley, although collections were made from only one of these sites. The other site where the species was recorded, but not collected, was 5 km NE of Huarán on the NW facing slope found to the immediate N of the prominent tower known by locals as "Kontorqayku", S 13°16′02.6″ W 72°01′12.9″, ca. 4300 m. This is the second species of *Lysipomia* to be noted as endemic to Peru (León & Lammers, 2006). However, the extent of its distribution is unclear as it is likely to have gone unnoticed by botanists until now and may occur in other areas of Peru.

Etymology. – The name refers to the beloved wife of the first author, Mitsy D.P.V. Sylvester, who was an invaluable help during vegetation surveys and was the first to spot this minute species.

Additional specimens examined. – PERU. Cuzco, Provincia Calca, Distrito Calca, grazed ground on the flat pampa 100 m W of Laguna Yanacocha, close to the settlements, 1.5 km E of Cancha Cancha village, Huarán, 4314 m, S 13°14′13.4″ W 72°01′06.3″, 22 Mar 2011, *S.P. Sylvester 823* (ASC, CUZ, MO, Z).



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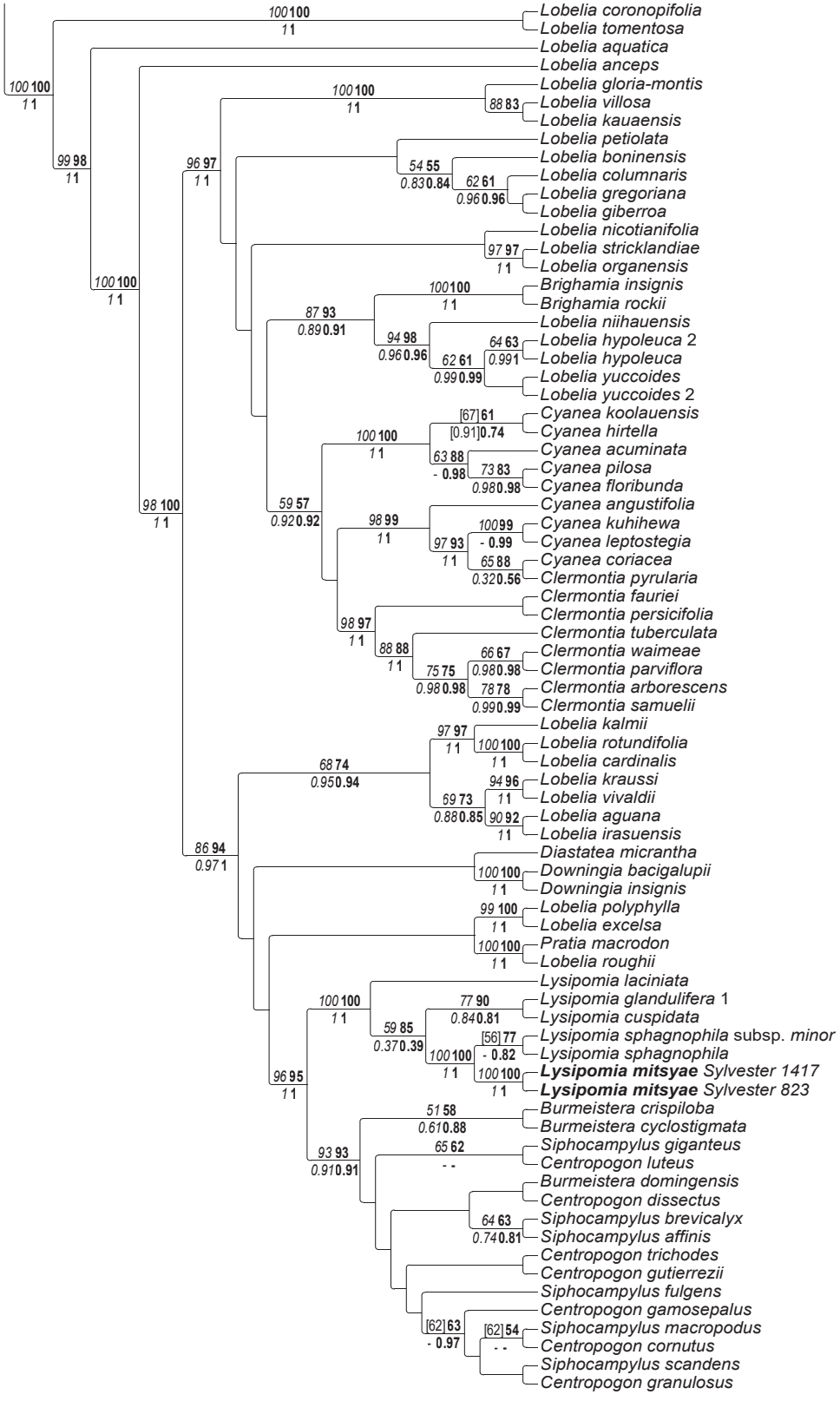

Fig. 3. Maximum likelihood phylogeny of the Lobelioideae, based on the *trnL-F* dataset, complemented with support values along the branches. Only values of maximum likelihood bootstrap support >50 are shown above the branches, while posterior probabilities >0.7 are shown below. The second value along a branch (in bold) corresponds to the SIC-approach. Numbers in [ ] refer to conflicts, i.e., the respective analysis resulted in a different "supported" branching pattern. A dash indicates no support for a specific analysis. If no support for a branch at all was obtained we resigned to show dashes.

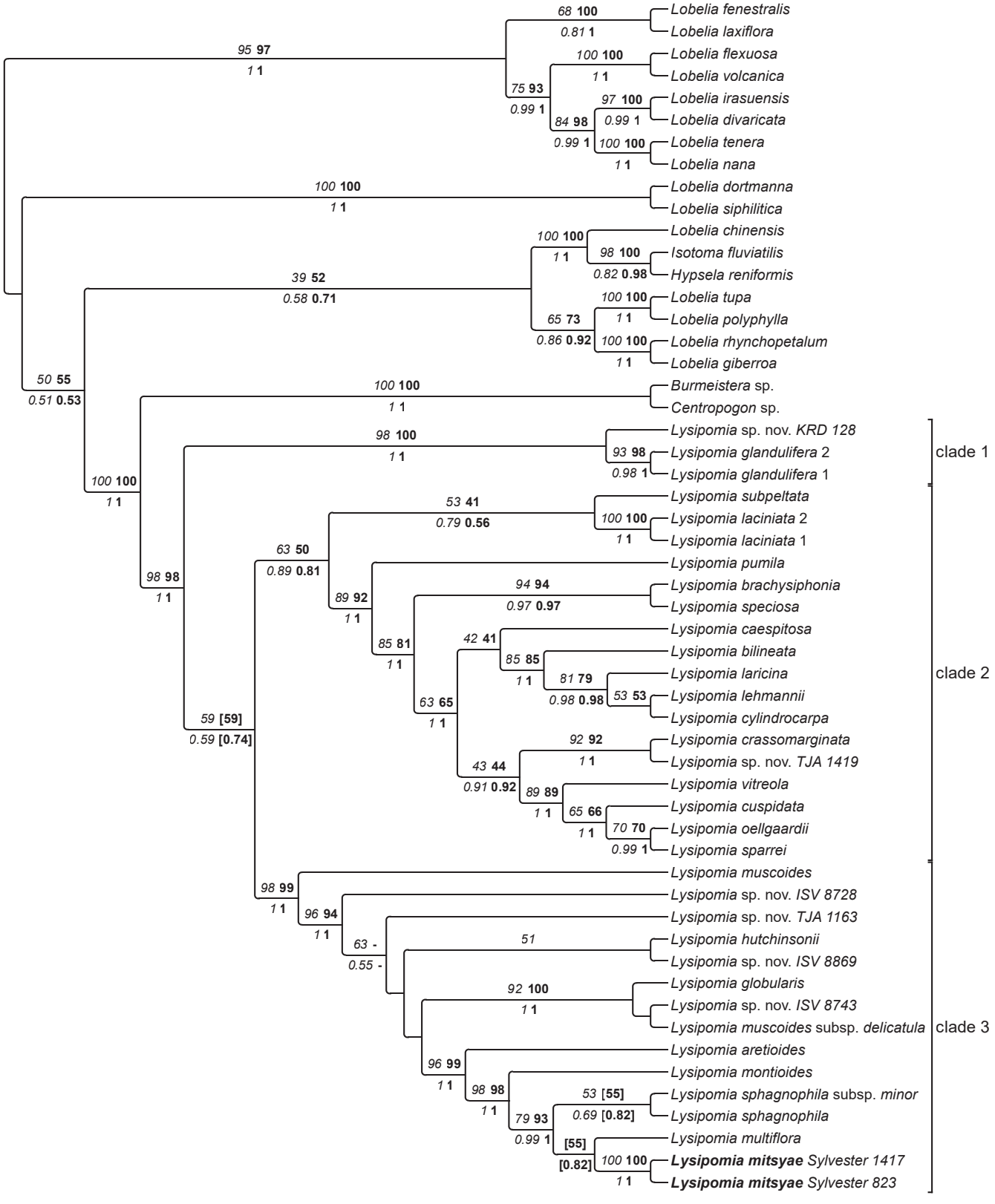

Fig. 4. Maximum likelihood phylogeny of *Lysipomia*, based on ITS data, complemented with support values along the branches. Only values of maximum likelihood bootstrap support >50 are shown above the branches, while posterior probabilities >0.7 are shown below. The second value along a branch (bold) corresponds to the SIC-approach. Numbers in [ ] refer to conflicts, i.e. the respective analysis resulted in a different "supported" branching pattern. A dash indicates no support for a specific analysis. If no support for a branch at all was obtained we resigned to show dashes.

Comments. — This species can be easily separated from all other members of the genus by its small stature, presence of persistent cotyledons, absence or small number of leaves, and small number of flowers, with all floral and vegetative structures significantly smaller compared with most other species of *Lysipomia*. There are a few species of *Lysipomia* with relatively small flowers that all belong to subgenus *Lysipomia*, e.g., *L. crassomarginata* (E.Wimm.) Jeppesen, *L. montioides* Kunth, *L. muscoides* Hook.f. and *L. sphagnophila*, but all these differ from *L. mitsyae* by being generally larger and cushion-forming with numerous leaves and flowers. The smallest member of the genus, apart from *L. mitsyae*, is likely to belong to the *L. sphagnophila* complex, with specimens described as forming cushions as small as 1 cm high and 1–1.5 cm in diameter with flowers 2–2.5 mm long (Jeppesen, 1981). *Lysipomia sphagnophila* is also the only other species of *L.* subg. *Rhizocephalum* Wedd. with a short-lived monocarpic habit (T. Ayers, pers. comm.), but it differs from *L. mitsyae* in overall larger size by at least an order of magnitude, with plants being more robust with numerous (usually 30–100) leaves measuring >6 mm long and numerous (often >50) flowers per plant, whilst lacking persistent cotyledons and staminodes (McVaugh, 1955; Jeppesen, 1981).

Molecular phylogenetic analyses. — The *trnL-F* Lobelioideae backbone dataset yielded 1288 characters, of which 92 positions were excluded due to a microsatellite region of tandem repeats, leaving 389 parsimony-informative (PI) positions. Phylogenetic analyses of this dataset clearly identified both unidentified specimens (with identical sequence data for both molecular markers) as a member of *Lysipomia* and sister to *L. sphagnophila* (both subsp. *sphagnophila* [*Sylvester 1885*, GenBank accession LN899774] and subsp. *minor* McVaugh [*Ayers 1392*, GenBank accession AF054943], according to T. Ayers, pers. comm.) with maximum support (Fig. 3). Although both species share several unique sequence characteristics such as various indels (>5 nt up to 18 nt), they are also conspicuously different (*P*-dist = 1.9%) with a distinguishing repeat (6 nt) in *L. sphagnophila* as well as a 5 nt indel in *L. mitsyae.* However, the monophyly of *L. sphagnophila* does not receive support, as a grade is indicated in the analyses without indel coding. A more detailed analysis of the intrageneric relationships based on the ITS data (1180 characters; PI sites = 264) converges to a similar scenario, with both *L. mitsyae* samples being genetically identical, while their relationship either to *L. multiflora* McVaugh or *L. sphagnophila* (including subsp. *minor*) have low support values (Fig. 4).

■ DISCUSSION

Morphological delimitation of *Lysipomia*. — The main characters that delimit *Lysipomia*, i.e., possession of capsules that dehisce via an apical operculum, unilocular ovaries with ventral parietal placentation, and non-resupinate flowers (McVaugh, 1955), were still retained in this minute member of the genus. Currently, the genus *Lysipomia* is further characterized as short herbs with leaves spirally arranged or in terminal rosettes. Flowers are either crowded and terminal or solitary in leaf axils, with a typically lobelioid corolla and androecium, the androecium containing 5 connate stamens (Wimmer, 1937, 1953; McVaugh, 1955; Jeppesen, 1981). Because of the differing morphology of *L. mitsyae* from other members of the genus, a recircumscription of the genus *Lysipomia* is required to include the following unique characters: common absence of true leaves, presence of persistent cotyledons, inflorescence emerging from the cotyledon axils, reduction of functional stamen number from 5 to 3, and presence of staminodes.

Persistent cotyledons, especially those forming the principal photosynthetic organs of a plant, and inflorescences emerging from the cotyledon axils, are characters rarely found in the plant kingdom (e.g., *Streptocarpus* Lindl. and *Monophyllaea* Benn. & R.Br.; Nishii & al., 2004) and further study should be done to elucidate the evolutionary developmental processes leading to these extraordinary traits. The reduced size of the flower has led, understandably, to this species being the first member of *Lysipomia* to exhibit reduction of functional stamen number with the two ventral anthers being replaced by staminodial flap-like appendages. Other members of *Lysipomia* and some lobelioid genera (e.g., *Centropogon*) have bristle-like appendages emerging from the ventral anthers and it is known that these appendages play a role in pollen dispersal, i.e., secondary pollen placement (Leins & Erbar, 2006), although loss of this character is common (e.g., *Burmeistera*).

Phylogenetic placement of *Lysipomia mitsyae*. — *Lysipomia mitsyae* was found to be a member of *Lysipomia* and nested within subgenus *Lysipomia*, which is characterized by small flowers with corollas that lack nectar guides (Ayers, 1999). *Lysipomia mitsyae* was tentatively placed in close relationship to *L. sphagnophila*, which includes subspecies previously considered the smallest members of the genus. ITS data also shows a close relationship to *L. multiflora* McVaugh, a robust cushion-forming perennial with a stout taproot and relatively large (7–9 mm long) flowers (McVaugh, 1955). However, as support values are low and, morphologically, *L. sphagnophila* bears more characteristics of *L. mitsyae*, it is the opinion of all authors that *L. sphagnophila* is more likely, among the species sampled, to be the sister species of *L. mitsyae*. *Lysipomia sphagnophila* is the only widespread species of subgenus *Lysipomia*, being found from Venezuela to Bolivia, and comprises many infraspecific taxa (McVaugh, 1955; Jeppesen, 1981). This suggests two likely scenarios for the evolution of *L. mitsyae*. First, one may hypothesize that *L. sphagnophila* is the progenitor or parent species from which *L. mitsyae* evolved. Second, it is possible that both shared a common, extinct ancestor. More exhaustive sampling of infraspecific taxa of *L. sphagnophila* is needed to test these hypotheses. In particular, from extensive field collections (S.P. Sylvester, unpub. data), herbarium work, and literature (McVaugh, 1955), we can ascertain that the *L. sphagnophila* found in the Cusco region is subsp. *sphagnophila* so it may be worth studying the relationships of *L. mitsyae* to this taxon in more detail.

Ecology of the world's smallest Campanulaceae. — The genus *Lysipomia* contains ca. 30–40 species endemic to the high Andes, inhabiting paramo and humid puna grasslands above 3000 m, with most species considered to be narrow endemics, often restricted to a single ridge or volcanic cone

(McVaugh, 1955; Ayers, 1997; Lammers, 2007; Price & Ayers, 2008). The genus has been found to have a moderately high net diversification rate and contributes to the high Andean paramo being considered "the world's fastest evolving and coolest biodiversity hotspot" (Madriñán & al., 2013). The majority of *Lysipomia* species are found in the northern Andes, extending from Venezuela to northern Peru, where the highest diversity is found on both sides of the Amotape-Huancabamba zone (Luteyn, 1999; Sklenář & al., 2011). Interestingly, *L. mitsyae* is found in fairly dry puna vegetation of southern Peru. Only four other species, *L. glandulifera*, *L. laciniata*, *L. pumila* and *L. sphagnophila*, are known from southern Peru and Bolivia but these occur in humid puna vegetation (Brako & Zarucchi, 1993; Jørgensen & al., 2014; Tropicos, 2015).

Lysipomia mitsyae grows in biological soil crusts formed by cyanobacteria and algae in high-elevation puna grasslands of the central Andes. The species thus appears to be adapted to a nutrient-poor, overgrazed, exposed habitat (Fig. 2B) where it has to face challenges of aluminium toxicity in the soils (Heitkamp & al., 2014) and mean daily temperature oscillations ranging by 24°C with daily mean minimum temperatures being −1.7°C (Heitkamp & al., 2014: 18). The climate is notably drier compared to sites where other members of *Lysipomia* occur (S.P. Sylvester, unpub. data) and may reflect adaptations of *L. mitsyae* to drier environments, possibly through its ephemeral lifecycle. The exceptionally small size of the plant, coupled with the harsh climate experienced by these high-elevation grasslands (for climatic data see Heitkamp & al., 2014: 18) opens up a plethora of questions regarding the physiological capacity of plants to survive in extreme environments. The discovery of this unusual plant should spur further research to discern what limitations govern alpine plant growth and survival.

Lysipomia is sister to *Burmeistera*, *Centropogon*, and *Siphocampylus* Pohl, which are large plants, sometimes even true trees, which is rare in Campanulaceae (Antonelli, 2008, 2009; Knox & al., 2008; Lagomarsino & al., 2014). The origin of the genus *Lysipomia*, thus, seems to be a case of dwarfism, likely caused by the extreme habitat of the high Andes, as observed in other plant genera (Körner, 2003). Our finding of *L. mitsyae* suggests that, within *Lysipomia*, this syndrome of dwarfism has proceeded to an absolute extreme. Argument could arise over whether this species is phenotypically, rather than genotypically, small and that its reduced size could be a response to drought, nutrient shortage, high light stress, or low temperatures (Körner & Pelaez Menendez-Riedl, 1989) and, under more favourable conditions, it would grow larger. Dwarfism has been documented from different alpine areas (e.g., Shinohara & Murakami, 2006; Körner, 2003) but, in the case of *L. mitsyae*, no larger specimens have been found, either at lower elevations close to the study area or in the local herbaria (CUZ, LPB, USM). It is thus likely, based on current knowledge, that this species is at its ecological optimum but that it occupies a very narrow ecological niche that is maintained by heavy grazing and harsh conditions and that, under more favourable conditions, it would be outcompeted by other forbs. This assumption is supported by how *L. mitsyae* was not noted in areas with reduced grazing which probably reflects competition by tussock grasses for light in less disturbed habitats. The likelihood that this species has its niche maintained by consistent heavy livestock grazing raises the question of its vulnerability to changing land use, should a less intensive grazing scheme be employed. On the other hand, it also raises the question as to the natural habitat of the species in the absence of human pastoral activities.

Could this be the world's smallest dicotyledonous plant? — Following a literature search of all species belonging to Campanulaceae (Wimmer, 1937, 1943, 1953, 1968; Jeppesen, 1981), none were found with a habit as diminutive as *L. mitsyae*. This exceptionally small species evidently holds the record for the world's smallest Campanulaceae and, quite possibly, the world's smallest eudicot. Within the eudicots, the current record for smallest species is held by *Arceuthobium minutissimum*, a leafless parasitic mistletoe measuring 2–5 mm, not including the haustoria (Datta, 1951). There are a number of other contenders for the title of smallest dicotyledonous species and, interestingly, the majority of these are found in the high-elevation grasslands and tundra of the Andes. The central and southern high Andes seem to be a "hotspot" for miniscule plants with the recent discovery of the aptly named *Viola lilliputana* Iltis & H.E.Ballard (Ballard & Iltis, 2012) from Peru, and miniscule members of *Oxalis* L., *Geranium* L., *Crassula* L., *Draba* L., etc. being mentioned by Körner (2003: 236) from Argentina. We also found other small annual species such as *Crassula closiana* (Gay) Reiche and *Cicendia quadrangularis* (Dombey ex Lam.) Griseb. growing alongside *L. mitsyae* on open, bare ground. However, none have thus far been found which are smaller than *L. mitsyae* in terms of height and overall habit, although a much more exhaustive literature search would be needed to verify the claim for a world record.

Leaving aside the question regarding which individual plant species might be smallest, perhaps more interesting is the fact that the smallest eudicots all have a size of 2–5 mm. Could it be that this is the minimum size physiologically possible for homoiohydric land plants, i.e., those that regulate and achieve homeostasis of cell and tissue water content? A similar situation is well known among warm-blooded vertebrates, where a minimum body mass of ca. 2 g, determined by physiological constraints of energy uptake and loss, has independently been reached in birds and mammals (Fons & al., 1997; Schuchmann, 1999; Norberg & Norberg, 2012). Among terrestrial plants, it is striking that homoiohydric plants do not become much smaller than 2 mm. This contrasts with species in which water transport and storage is less important. In the aquatic realm, where water is not limiting, examples of much smaller plants are *Wolffia*, among the angiosperms, and the even smaller aquatic algae. On land, poikilohydric plants, i.e., those that have passive water uptake and loss, include bryophytes less than 1 mm in size (e.g., epiphyllous Lejeuneaceae) and much smaller minute algae. For homoiohydric plants that need to take up, transport, and store water, there may be physical and anatomical constraints that would not allow a plant to have roots, stems and leaves below a certain minimum size. Minute plants, such as *L. mitsyae*, may have reached this limit and, as such, might provide interesting insights into the limitations of plant size at the lower end of the size spectrum.

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Appendix 1. Voucher specimens used in this study.

GenBank accession numbers for the downloaded *trnL-F* sequences of the Campanulaceae backbone dataset (species, accession number).

Adenophora remotiflora (Siebold & Zucc.) Miq., EF088693; ***Asyneuma campanuloides*** (M.Bieb. ex Sims) Bornm., FJ426570; ***A. canescens*** (Waldst.) Griseb. & Schenk, FJ426567; ***A. limonifolium*** (L.) Janch., FJ426571; ***A. lobelioides*** (Willd.) Hand.-Mazz., EF088695 & FJ426568; ***A. pichleri*** (Vis.) D.Lakušić & F.Conti, FJ426569; ***A. trichocalycinum*** (Ten.) K. Malý, FJ426566; ***Brighamia insignis*** A.Gray, DQ356189; ***B. rockii*** H.St.John, DQ285140; ***Burmeistera crispiloba*** Zahlbr., DQ285164; ***B. cyclostigmata*** Donn.Sm., DQ356213; ***B. domingensis*** Jeppesen, DQ356214; ***Campanula aparinoides*** Pursh, EF088702; ***C. cymbalaria*** Sibth. & Sm., EF088715; ***C. debarensis*** Rech.f., FJ426575; ***C. decumbens*** A.DC., EF088716; ***C. divaricata*** Michx., EF088718; ***C. erinus*** L., EF088720; ***C. fastigiata*** Dufour ex Schult., EF088721; ***C. garganica*** Ten., FJ426581; ***C. isophylla*** Moretti, FJ426583; ***C. lusitanica*** Loefl., EF088733; ***C. moravica*** (Spitzn.) Kovanda, EF088740; ***C. persicifolia*** L., FJ426573; ***C. portenschlagiana*** Schult., FJ426587; ***C. prenanthoides*** Durand, EF088748; ***C. pterocaula*** Hausskn., EF088751; ***C. pyramidalis*** Gilib., EF088754; ***C. rapunculus*** L., EF088758; ***C. rotundifolia*** L., EF088759; ***C. uniflora*** L., FJ426574; ***C. versicolor*** Andrews, FJ426591; ***Campanulastrum americanum*** (L.) Small, EF088776; ***Centropogon cornutus*** (L.) Druce, DQ356226; ***C. dissectus*** E.Wimm., DQ356215; ***C. gamosepalus*** Zahlbr., DQ356225; ***C. granulosus*** C.Presl, DQ356220; ***C. gutierrezii*** (Planch. & Oerst.) E.Wimm., DQ285165; ***C. luteus*** E.Wimm., DQ356219; ***C. trichodes*** E.Wimm., DQ356217; ***Clermontia arborescens*** (H.Mann) Hillebr., DQ285141; ***C. fauriei*** H.Lév., DQ285142; ***C. parviflora*** Gaudich. ex A.Gray, DQ285171; ***C. persicifolia*** Gaudich., KC460649; ***C. pyrularia*** Hillebr., KC460650; ***C. samuelii*** F.B.Forbes, KC460651; ***C. tuberculata*** C.N.Forbes, KC460652; ***C. waimeae*** Rock, KC460653; ***Cyanea acuminata*** (Gaudich.) Hillebr., DQ285144; ***C. angustifolia*** (Cham.) Hillebr., DQ356173; ***C. coriacea*** Hillebr., DQ285145; ***C. floribunda*** E.Wimm., DQ285173; ***C. hirtella*** Hillebr., DQ285175; ***C. koolauensis*** Lammers, Givnish & Sytsma, DQ356193; ***C. kuhihewa*** Lammers, DQ285177; ***C. leptostegia*** A.Gray, DQ285172; ***C. pilosa*** A.Gray, DQ285174; ***Diastatea micrantha*** (Kunth) McVaugh, DQ356203; ***Downingia bacigalupii*** Weiler, DQ356183; ***D. insignis*** Greene, DQ356185; ***Legousia hybrida*** (L.) Delarbre, EF088783; ***Lobelia aguana*** E.Wimm., DQ356176; ***L. anceps*** L.f., DQ356184; ***L. aquatica*** Cham., DQ356182; ***L. boninensis*** Koidz., DQ285157; ***L. cardinalis*** L., DQ285168; ***L. columnaris*** Hook.f., DQ285158; ***L. coronopifolia*** L., DQ356181; ***L. excelsa*** Bonpl., DQ285159; ***L. giberroa*** Hemsl., DQ285160; ***L. gloria-montis*** Rock, DQ285148; ***L. gregoriana*** Baker f., DQ356187; ***L. hypoleuca*** Hillebr., DQ285149 & DQ356191; ***L. irasuensis*** Planch. & Oerst., DQ356175; ***L. kalmii*** L., EF126736; ***L. ×kauaiensis*** (A.Gray) A.Heller, DQ285150; ***L. kraussi*** Graham, DQ356179; ***L. nicotianifolia*** Roth ex Schult., DQ285161; ***L. niihauensis*** H.St.John, DQ285151; ***L. organensis*** Gardner, DQ285162; ***L. petiolata*** Hauman, DQ285163; ***L. polyphylla*** Hook. & Arn., DQ356177; ***L. rotundifolia*** Juss. ex A.DC., DQ356178; ***L. roughii*** Hook.f., EF126737; ***L. stricklandiae*** Gilliland, DQ356186; ***L. tomentosa*** L.f., DQ356180; ***L. villosa*** (Rock) H.St.John & Hosaka, DQ285176; ***L. vivaldii*** Lammers & Proctor, DQ285167; ***L. yuccoides*** Hillebr., DQ285152 & DQ356190; ***Lysipomia cuspidata*** McVaugh, DQ356198; ***L. sphagnophila*** Griseb. ex Wedd., DQ356197; ***Petromarula pinnata*** A.DC., FJ426585; ***Physoplexis comosa*** (L.) Schur, FJ426586; ***Phyteuma globulariifolium*** Sternb. & Hoppe, FJ426582; ***P. spicatum*** L., EF088787; ***Pratia macrodon*** Hook.f., AY568753 & AY568742; ***Siphocampylus affinis*** (Mirb.) McVaugh, DQ356223; ***S. brevicalyx*** E.Wimm., DQ356224; ***S. fulgens*** Lebas, DQ356216; ***S. giganteus*** (Cav.) G.Don, DQ356222; ***S. macropodus*** G.Don, DQ356221; ***S. scandens*** (Kunth) G.Don, DQ356218; ***Symphyandra hofmannii*** Pantan., EF088727; ***Trachelium caeruleum*** L., EF088791.

Generated *trnL-F* sequences (species, isolate, voucher (herbarium; duplicates in), accession number).

Lysipomia glandulifera (Schltdl. ex Wedd.) Schltr. & E.Wimm., ED1101, *Sylvester 1891* (Z; LPB), LN828289*; ***L. laciniata*** A.DC., ED1100, *Sylvester 213* (Z; CUZ), LN828288*; ***L. mitsyae*** Sylvester & D.Quandt, ED884, *Sylvester 1417* (Z; CUZ, LPB), LN828287*; ***L. mitsyae***, ED1186, *Sylvester 823* (Z; CUZ, LPB), LN899775*; ***L. sphagnophila***, ED1193, *Sylvester 1885* (Z; CUZ, LPB), LN899774.

GenBank accession numbers for the downloaded ITS1 & 2 sequences of the *Lysipomia* backbone dataset (species, accession number).

Hypsela reniformis (Kunth) C.Presl, AF054941; ***Isotoma fluviatilis*** (R.Br.) F.Muell. ex Benth., AY644648; ***Lobelia chinensis*** Lour., KM051440; ***L. divaricata*** Hook. & Arn., AY362764; ***L. dortmanna*** L., EU219388; ***L. fenestralis*** Cav., AY350634; ***L. flexuosa*** (C.Presl) A.DC., AY350626; ***L. giberroa***, EU219380;; ***L. irasuensis***, AY362765; ***L. laxiflora*** Kunth, AY350631; ***L. nana*** Kunth, AY350629; ***L. polyphylla***, AY350633; ***L. rhynchopetalum*** (Hochst. ex A.Rich.) Hemsl., FJ664109; ***L. siphilitica*** L., DQ006015; ***L. tenera*** Kunth, AF054938 AF054938; ***L. tupa*** L., AY350632; ***L. volcanica*** T.J.Ayers, AY350625; ***Lysipomia aretioides*** Kunth, AF054964; ***L. bilineata*** McVaugh, AF054963; ***L. brachysiphonia*** E.Wimm., AF054962; ***L. caespitosa*** T.J.Ayers, AF054961; ***L. crassomarginata*** (E. Wimm.) Jeppesen, AF054960; ***L. cuspidata*** McVaugh, AF054959; ***L. cylindrocarpa*** T.J. Ayers, AF054958; ***L. muscoides*** subsp. ***delicatula*** McVaugh, AF054957; ***L. glandulifera***, AF054956; ***L. globularis*** E.Wimm., AF054955; ***L. hutchinsonii*** McVaugh, AF054954; ***L. laciniata* 1**, AF054953; ***L. laricina*** E.Wimm., AF054952; ***L. lehmannii*** Hieron. ex Zahlbr., AF054951; ***L. montioides*** Kunth, AF054950; ***L. multiflora*** McVaugh, AF054949; ***L. muscoides*** Hook. f., AF054948; ***L. oellgaardii*** Jeppesen, AF054947; ***L. pumila*** (Wedd.) E. Wimm., AF054946; ***L.* sp. nov.**, *Kristin Rae Dotti 128*, AF054940; ***L.* sp. nov.**, *Tina J. Ayers 1163*, AF054966; ***L.* sp. nov.** *Tina J. Ayers 1419*, AF054968; ***L.* sp. nov.** *Isidoro Sanchez Vega 8728*, AF054965; ***L.* sp. nov.**, *Isidoro Sanchez Vega 8743*, AF054967; ***L.* sp. nov.**, *Isidoro Sanchez Vega 8869*, AF054969; ***L. sparrei*** Jeppesen, AF054945; ***L. speciose*** T.J.Ayers, AF054944; ***L. sphagnophila***, AF054943; ***L. subpeltata*** McVaugh, AF054942; ***L. vitreola*** McVaugh, AF054939.

Generated ITS sequences (species, isolate, voucher (herbarium; duplicates in), accession number).

Burmeistera H.Karst. & Triana **sp. 1**, ED1194, CR-0-BAS-239/2014 W S, LN899772; ***Centropogon*** C.Presl **sp. 1**, ED1195, BO0Z-20130740, LN899773; ***Lysipomia glandulifera***, ED1101, *Sylvester 1891* (Z; LPB), LN828292; ***L. laciniata***, ED1100, *Sylvester 213* (Z; CUZ), LN828291; ***L. mitsyae***, ED884, *Sylvester 1417* (Z; CUZ, LPB), LN828290; ***L. mitsyae***, ED1186, *Sylvester 823* (Z; CUZ, LPB), LN899770; ***L. sphagnophila***, ED1193, *Sylvester 1885* (Z; CUZ, LPB), LN899771.